Pergamon

0197-0186(94)E0043-S

Neurochem. Int. Vol. 25, No. 2, pp. 155–159, 1994

0197-0186/94 $7.00+0.00

ASYMMETRIC CROSS-SENSITIZATION TO THE LOCOMOTOR STIMULANT EFFECTS OF PHENCYCLIDINE AND MK-801*

XIAOJUAN XU and EDWARD F. DOMINO†
Department of Pharmacology, University of Michigan, Ann Arbor, MI 48109-0626, U.S.A.

(Received 4 November 1993; accepted 14 February 1994)

Abstract—Chronic administration of a psychomotor stimulant has been shown to produce progressively enhanced effects, a phenomenon called "reverse tolerance" or sensitization. Sensitization which develops to the psychomotor stimulant effect of a drug generalizes to drugs with similar neurochemical mechanisms of action, a phenomenon called cross-sensitization. The present study compared the psychomotor stimulant effects of phencyclidine and MK-801, examined the effects of the daily injection of phencyclidine and MK-801 on locomotor activity and investigated whether reciprocal cross-sensitization occurred between phencyclidine and MK-801. Adult female Sprague–Dawley rats were used. Their locomotor activity was measured automatically for a 2 h period following drug injection. Phencyclidine and MK-801 both increased locomotor activity. Four daily injections of phencyclidine in a dose of 3.2 mg/kg i.p., or MK-801 in a dose of 0.32 mg/kg i.p., produced sensitization to locomotor activity. Moreover, MK-801 sensitized rats showed cross-sensitization to phencyclidine. However, phencyclidine sensitized rats did not show cross-sensitization to MK-801. This finding suggests that there are significant differences in the neurochemical mechanisms underlying phencyclidine-induced and MK-801-induced sensitization. Phencyclidine sensitization may not be mediated by NMDA receptors.

Phencyclidine (PCP) is known to inhibit striatal dopamine (DA) reuptake, facilitate its release and affect its synthesis and metabolism in rodents (Johnson and Jones, 1990). In addition, PCP also has anticholinergic properties (Finnegan *et al.*, 1976; Murray, 1983), interacts with the σ-receptor (Quirion *et al.*, 1988) and affects brain norepinephrine (NE) and serotonin (5-HT) (Johnson and Jones, 1990). Although PCP exerts its effects through several neurotransmitter systems, understanding of its mechanism of action has been greatly enhanced since PCP was observed to selectively reduce the excitatory actions of glutamate on spinal neurons that were mediated by *N*-methyl-D-aspartate (NMDA) receptors (Anis *et al.*, 1983).

The NMDA receptor mediates ion fluxes through a channel permeable to Na^+, K^+, and Ca^{2+}. The ion flux is voltage dependent and is gated by Mg^{2+}. Phencyclidine acts at a distinct site within the channel itself. The mechanism by which PCP inhibits NMDA-mediated responses is noncompetitive (Harrison and Simmonds, 1985; Snell and Johnson, 1986). Although it seems that the anticonvulsive and neuroprotective effects are the results of blockade of ionic conductance through the NMDA-operated ion channel, the neural bases of the psychotomimetic properties of PCP remain unclear.

Receptor binding studies indicate that PCP binds to at least two major sites in brain tissue: a PCP site that is associated with NMDA receptors and a σ-receptor site that may modulate the release of a number of neurotransmitters (McCullough and Salamone, 1992). The acute locomotor stimulant effects of PCP and SKF-10,047, a PCP as well as σ ligand are similar with reciprocal cross-sensitization (Iwamoto, 1986; Greenberg and Segal, 1986). These findings suggest that the neural basis of the psychotomimetic effects of PCP may, at least in part, be the result of an interaction with σ-receptors. Phencyclidine also binds to a channel site associated with NMDA receptors, which may also account for its psychotomimetic effect.

*Supported in part by NIDA grant DA-01531.
†Author to whom all correspondence should be addressed: Dr E.F. Domino, Department of Pharmacology, University of Michigan, M6414 Medical Science Builidng I, Ann Arbor, MI 48109-0626, U.S.A.
Part of this work was presented at the *Excitatory Amino Acid Neurotransmission* Meeting, Marseilles, France, 29 August–2 September, 1993, organized by Max Recasens.

Numerous studies suggest that PCP and MK-801 act at the same channel associated with the NMDA receptor (Anis *et al.*, 1983; Fagg, 1987; Harrison and Simmonds, 1985). MK-801, a noncompetitive antagonist at the NMDA receptor (Wong *et al.*, 1986) with little or no affinity for the σ-receptor (Wong *et al.*, 1988), has become an important pharmacological tool for studying NMDA receptor function. To investigate whether antagonism of NMDA receptors by PCP accounts for its psychotomimetic effect, comparisons between the locomotor stimulant effects of PCP and MK-801 may shed light on this issue. The present study had three objectives. The first was to compare the locomotor stimulant effects of PCP and MK-801 after acute administration to rats. The second was to determine if sensitization develops after repeated administration of either PCP or MK-801. Third, whether PCP and MK-801 show cross-sensitization.

EXPERIMENTAL PROCEDURES

Subjects

Adult female Sprague–Dawley rats, weighing 190–270 g, were used because they are known to be more susceptible than male rats to behavioral sensitization. The animals were allowed at least 1 week of acclimatization to the animal facilities. During this time, as well as during the subsequent experimental period, the rats were housed two or three per cage with free access to food and water in a rodent room with constant temperature, humidity and a 12 h light–dark cycle (0700–1900 light).

Apparatus

Locomotor activity of each animal was measured with the Digiscan "Micro" system consisting of four mounting frames and one analyzer (Omnitech Electronics, Columbus, OH 43228). A mounting frame contained two parallel panels, one photocell panel with 16 infrared light beams spaced 2.54 cm apart and one light beam detector panel. Each rat was placed in a transparent Plexiglas cage ($46 \times 24 \times 18$ cm) within a mounting frame located in a sound dampened chamber. The Digiscan system detected locomotor activity by counting interruptions of consecutive light beams caused by the animal moving from one location to another. Data were automatically recorded and processed by the analyzer and further transferred to and stored on a Macintosh IIsi computer.

Drugs

Phencyclidine (National Institute on Drug Abuse, Rockville, MD 20857) was dissolved in dilute HCl and saline (0.9% NaCl) solution. The drug was then neutralized with NaOH to give a final pH of approx. 6.4. Phencyclidine was administered i.p. once daily for four consecutive days in a dose of 3.2 mg/kg. (+)-MK-801 hydrogen maleate (Research Biochemicals, Inc., Natick, MA 01760) was dissolved in saline. A dose of 0.32 mg/kg of MK-801 was administered i.p. once daily for four consecutive days.

Procedure

Rats were randomly divided into three groups. Groups were injected with either saline, PCP or MK-801 and tested in the Digiscan system daily for four consecutive days. Locomotor activity was assessed immediately following injection of each agent. Each animal's activity was monitored continuously for the next 120 min. Data were accumulated in 10 min blocks throughout the 120 min period.

On the fifth day, half of the daily saline-treated rats received PCP and the other half received MK-801. Daily PCP-treated rats received MK-801 and daily MK-801-treated rats received PCP. Rats were tested in the Digiscan system immediately after i.p. injection of 3.2 mg/kg PCP or 0.32 mg/kg MK-801. Motor activity was monitored the same as before.

RESULTS

Acute administration of either 3.2 mg/kg PCP or 0.32 mg/kg MK-801 produced marked increases in locomotor activity over the 2 h sessions compared with saline injection (Fig. 1). A one-way ANOVA with multiple comparisons on total activity counts showed that the increases in locomotor activity induced by both agents was significant over the 2 h session ($F(2,18) = 35.453$, $P < 0.001$).

While daily saline injection decreased locomotor activity, daily administration of either 3.2 mg/kg PCP or 0.32 mg/kg MK-801 resulted in an enhanced locomotor stimulant effect over four consecutive days (Fig. 2). The difference in locomotor activity between the first and fourth injection of PCP was subjected to a correlated t-test, which indicated a statistically significant increase ($P < 0.01$). There were no significant differences between days 2, 3 and 4 for PCP. The difference in locomotor activity following the first

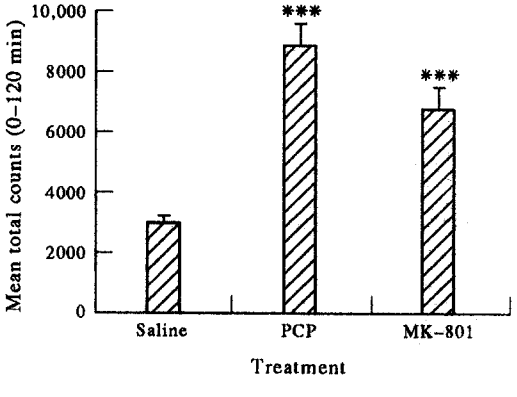

Fig. 1. Locomotor activity of rats treated with either saline or PCP or MK-801 over the 2 h session on day 1. Each bar represents the total activity counts in 120 min ± SE for 5–10 rats. ***$P < 0.001$ compared with locomotor activity of rats treated with saline.

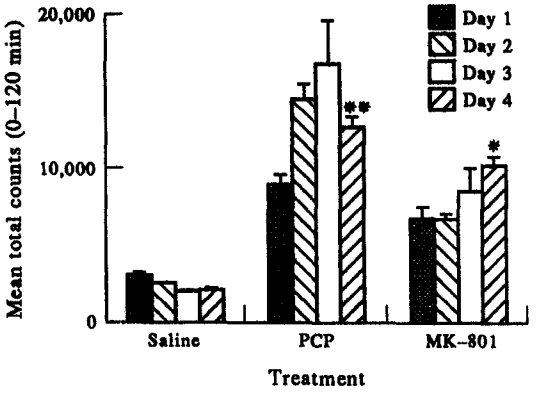

Fig. 2. Locomotor activity of rats treated with either saline or PCP or MK-801 over 4 consecutive days. Each bar represents the mean total activity counts in 120 min ± SE for 5–10 rats. $*P < 0.05$, $**P < 0.01$ compared with related locomotor activity on day 1.

compared to the fourth injection of MK-801 was also statistically significant ($P < 0.05$).

The locomotor activity of daily PCP and MK-801 were analyzed further. The data for each 30 min subsession over the 2 h session on day 1 and day 4 are shown in Fig. 3. Daily PCP produced a parallel upward shift in locomotor response over the 2 h session without changing its onset and peak action (Fig. 3: left panel). Daily MK-801 also induced a upward shift in the locomotor response. However, the shift was not parallel and the peak of MK-801 was also shifted upward after four daily injections (Fig. 3: right panel).

Despite some similarities between PCP and MK-801 in their acute and subchronic effects, asymmetric cross-sensitization occurred between the two. The PCP-induced locomotor response was significantly enhanced after four daily MK-801 injections when compared to that after four daily saline injections, whereas the MK-801-stimulated locomotor response did not show a significant change after four daily PCP injections (Fig. 4).

DISCUSSION

Acute administration of PCP to rats increases locomotor activity, rearing, stereotypy and ataxia (Greenberg and Segal, 1985; Iwamoto, 1986; Castellani and Adams, 1981). A somewhat similar motor syndrome with more ataxia is produced by the acute administration of MK-801 (Koek *et al.*, 1988; Hoffman, 1992). In the present study, both PCP and MK-801 significantly increased locomotor activity, i.e., from one location to another. However, when rats were observed through a one-way mirror, only the former produced stereotypies and rearing in the doses used. Therefore, stereotypy and rearing were not suitable behavioral endpoints for this study. In a preliminary study, 1.8 mg/kg PCP and 0.18 mg/kg MK-801 were used. A dose of 1.8 mg/kg PCP produced much less increase in locomotor activity than 3.2 mg/kg PCP, while 0.18 mg/kg MK-801 produced a greater increase in locomotor activity than 0.32 mg/kg MK-801. However, at both doses, PCP produced much more locomotor stimulation than MK-801. Because 0.18 mg/kg MK-801 did not produce sensitization to locomotor activity in the previous study (Xu and Domino, 1994), the present study employed 0.32 mg/kg MK-801. The present study was also limited to a 5 day period.

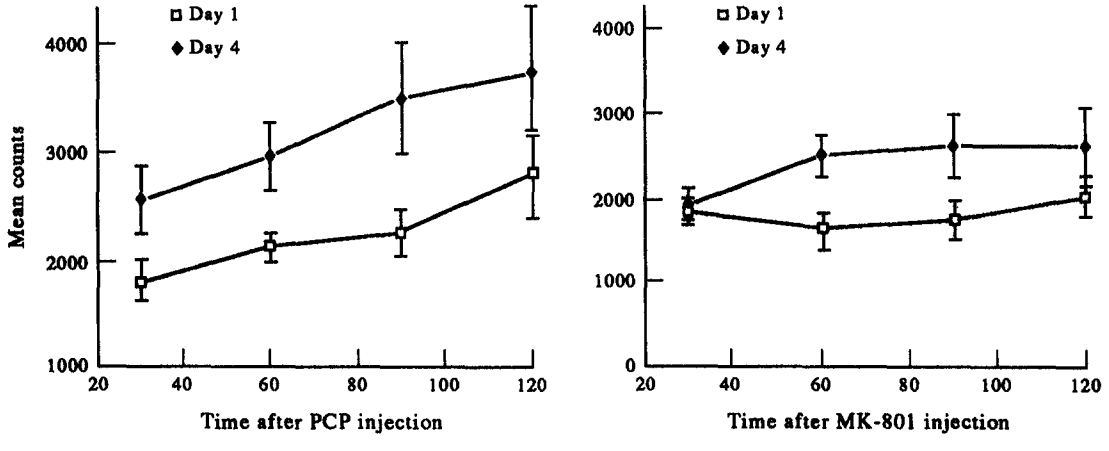

Fig. 3. Locomotor activity of rats treated with PCP (left panel) and rats treated with MK-801 (right panel) in 30 min block over the 2 h sessions on day 1 and day 4. Each point represents the mean activity counts in 30 min ± SE for 5–6 rats.

Future studies should be done using longer periods of subchronic treatments.

Chronic administration of PCP results in an enhanced locomotor stimulant effect (Castellani and Adams, 1981; Greenberg and Segal, 1985, 1986; Iwamoto, 1986; Stergeon *et al.*, 1982). Chronic administration of MK-801 also sensitized rats to the locomotor stimulant effects of MK-801 (Wolf and Khansa, 1991; Wolf *et al.*, 1993). The results of the present study confirmed that chronic administration of PCP or MK-801 produce sensitization to their locomotor stimulant effects. However, the neural mechanisms underlying PCP sensitization may differ from those of MK-801. Four daily injections of PCP produced a parallel upward shift in locomotor response pattern over the 2 h session without changing the onset and the peak of its action (Fig. 3: left panel), while daily MK-801 injections produced a nonparallel upward shift in its locomotor response pattern, with the peak action shifted to the left (Fig. 3: right panel). Phencyclidine and MK-801 sensitization may be due to changes in the rate of their biotransformation. For example PCP is a suicide inhibitor of its own *P*450 metabolism (Osawa and Coon, 1989). Nevertheless, PCP-induced sensitization has been suggested to be due to a pharmacodynamic mechanism (Nabeshima *et al.*, 1987). Whether sensitization induced by both agents is due to pharmacokinetic factors needs to be investigated further.

Acute injection of MK-801 in a dose of 0.32 mg/kg was observed through a one-way mirror to produce severe ataxia, while acute 3.2 mg/kg PCP produced mild ataxia (data not shown). No changes in ataxia were observed with daily injections of MK-801 or PCP, which is in agreement with several other studies (Greenberg and Segal, 1986; Leccese *et al.*, 1986; Smith *et al.*, 1981). Therefore, sensitization induced by PCP or MK-801 may not be due to a pharmacokinetic factor because a change in all behaviors induced by the drugs would be expected if pharmacokinetic factors played a role in sensitization.

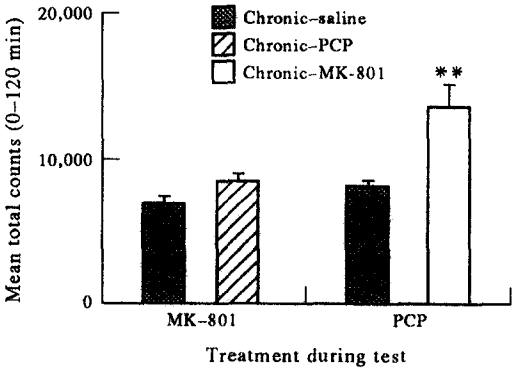

Fig. 4. Phencyclidine- and MK-801-induced locomotor activity after chronic pretreatment with saline, PCP or MK-801 respectively. Each bar represents the mean total activity counts in 120 min ± SE for 5–6 rats. $^{**}P < 0.01$ compared with locomotor activity of chronic-saline rats.

While sensitization to the effects of daily injection of PCP or MK-801 has previously been reported, this study is the first to examine their possible cross-sensitization. Both PCP and MK-801 enhanced locomotor activity and produced sensitization to locomotor activity after daily injection. However, an asymmetric cross-sensitization between PCP and MK-801 was observed. Phencyclidine-induced locomotor activity was enhanced after daily MK-801 injection, whereas MK-801-induced locomotor activity did not change after daily PCP injection. These results suggest that there are important differences in the neuronal mechanisms underlying repeated administration of both drugs. Because PCP sensitized rats did not show cross-sensitization to MK-801, it is unlikely that NMDA receptors play a major role in the development of PCP sensitization. Reciprocal cross-sensitization of locomotion is present between PCP and *N*-allylnormetazocine (NANM), a σ ligand (Greenberg and Segal, 1986; Iwamoto, 1986). Thus PCP-induced sensitization may be mediated mainly by an interaction with σ but not NMDA receptors. In another study, daily injections of 0.18 mg/kg of MK-801 were administered 30 min before 3.2 mg/kg PCP. The rats did not show sensitization to MK-801, but showed sensitization to PCP (Xu and Domino, 1994). In that study, MK-801 occupied NMDA receptor channels without producing sensitization, yet PCP still produced sensitization. This further suggests that the development of PCP sensitization does not require an interaction with the NMDA receptor complex. Future studies should compare σ binding correlates of different σ ligands to establish a role of σ receptors in this phenomenon.

In conclusion, although both PCP and MK-801 enhanced locomotor activity, the development of sensitization to the former showed a different pattern than to the latter. Furthermore, an asymmetric cross-sensitization occurred between PCP and MK-801. Phencyclidine sensitized animals did not show cross-sensitization to MK-801, whereas MK-801 sensitized animals showed cross-sensitization to PCP.

REFERENCES

Anis N. A., Berry S. C., Burton N. and Lodge D. (1983) The dissociative anesthetics ketamine and phencyclidine

selectively reduce excitation of central mammalian neurons by *N*-methyl-D-aspartate. *Br. J. Pharmac.* **79,** 565–574.

Castellani S. and Adams P. M. (1981) Acute and chronic phencyclidine effects on locomotor activity, stereotypy and ataxia in rats. *Eur. J. Pharmac.* **73,** 143–154.

Fagg G. E. (1987) Phencyclidine and related drugs bind to the activated *N*-methyl-D-aspartate receptor-channel complex in rat brain membranes. *Neurosci. Lett.* **76,** 221.

Finnegan K., Kanner M. and Meltzer H. Y. (1976) Phencyclidine-induced rotational behavior in rats with nigrostriatal lesions and its modulation by dopaminergic and cholinergic agents. *Pharmac. Biochem. Behav.* **5,** 651–660.

Greenberg B. D. and Segal D. S. (1985) Acute and chronic behavioral interactions between phencyclidine (PCP) and amphetamine: evidence for a dopaminergic role in some PCP-induced behaviors. *Pharmac. Biochem. Behav.* **23,** 99–105.

Greenberg B. D. and Segal D. S. (1986) Evidence for multiple opiate receptor involvement in different phencyclidine-induced unconditioned behaviors in rats. *Psychopharmacology* **88,** 44–53.

Harrison N. L. and Simmonds M. A. (1985) Quantitative studies on some antagonists of *N*-methyl-D-aspartate in slices of rat cerebral cortex. *Br. J. Pharmac.* **84,** 381–391.

Hoffman D. C. (1992) Typical and atypical neuroleptics antagonize MK-801-induced locomotion and stereotypy in rats. *J. Neural Transm.* **89,** 1–10.

Iwamoto E. T. (1986) Comparison of the pharmacologic effects of *N*-allynormetazocine and phencyclidine: sensitization, cross-sensitization and opioid antagonist activity. *Psychopharmacology* **89,** 221–229.

Johnson K. M. and Jones S. M. (1990) Neuropharmacology of phencyclidine: basic mechanisms and therapeutic potential. *Ann. Rev. Pharmac. Toxic.* **30,** 707–750.

Koek W., Woods J. H. and Winger G. D. (1988) MK-801, a proposed noncompetitive antagonist of excitatory amino acid neurotransmission produces phencyclidine-like behavioral effects in pigeons, rats and rhesus monkeys. *J. Pharmac. exp. Ther.* **245,** 969–974.

Leccese A. P., Marquis K. L., Mattia A. and Moreton J. E. (1986) The anticonvulsant and behavioral effects of phencyclidine and ketamine following chronic treatment in rats. *Behav. Brain Res.* **22,** 257–264.

McCullough L. D. and Salamone J. D. (1992) Increases in extracellular dopamine levels and locomotor activity after direct infusion of phencyclidine into the nucleus accumbens. *Brain Res.* **577,** 1–9.

Murray T. F. (1983) A comparison of phencyclidine with other psychoactive drugs on cholinergic dynamics on the rat brain. In: *Phencyclidine and Related Arylcyclohexylamines: Present and Future Applications* (Kamenka J. M., Domino E. F. and Geneste P., eds), pp. 547–561, NPP Books, P.O. Box 1491, Ann Arbor, MI 48106, U.S.A.

Nabeshima T., Fukaya H., Yamaguchi K. and Ishikawa K. (1987) Development of tolerance and supersensitivity to phencyclidine in rats after repeated administration of phencyclidine. *Eur. J. Pharmac.* **135,** 23–33.

Osawa Y. and Coon M. J. (1989) Selective mechanism-based inactivation of the major phenobarbital-inducible *P*-450 cytochrome from rabbit liver by phencyclidine and its oxidation product, the iminium compound. *Drug Metab. Disp.* **17,** 7–13.

Qurion R., Chiceportiche R., Contreras P. C., Johnson K. M., Lodge D., Tam S. W., Woods J. M. and Zukin S. R. (1988) Classification of nomenclature of phencyclidine and sigma receptor sites. In: *Sigma and Phencyclidine-Like Compounds as Molecular Probes in Biology* (Domino E. F. and Kamenka J.-M., eds.), pp. 601–606. NPP Books, P.O. Box 1491, Ann Arbor, MI 48106, U.S.A.

Smith R. D., Biggs C., Vroulis G. and Brinkman S. (1981) Effects of chronic administration of phencyclidine on stereotyped and ataxic behaviors in rats. *Life Sci.* **28,** 1163–1174.

Snell L. D. and Johnson K. M. (1986) Characterization of the inhibition of excitatory amino acid-induced neurotransmitter release in the rat striatum by phencyclidine-like drugs. *J. Pharmac. exp. Ther.* **238,** 938–946.

Stergeon R. D., Fessler R. G., London S. F. and Meltzer H. Y. (1982) Behavioral effects of chronic phencyclidine administration in rats. *Psychopharmacology* **76,** 52–56.

Wolf M. E. and Khansa M. R. (1991) Repeated administration of MK-801 produces sensitization to its own locomotor stimulant effects but blocks sensitization to amphetamine. *Brain Res.* **562,** 164–168.

Wolf M. E., White F. J. and Hu X. T. (1993) Behavioral sensitization to MK-801 (dizocilpine): neurochemical and electrophysiological correlates in the mesoaccumbens dopamine system. *Behav. Pharmac.* **4,** 429–442.

Wong E. H. F., Kemp J. A., Priestley T., Knight A. R., Woodruff G. N. and Iversen L. L. (1986) The anticonvulsant MK-801 is a potent *N*-methyl-D-aspartate antagonist. *Proc. Natn. Acad. Sci. U.S.A.* **83,** 7104–7108.

Wong E. H. F., Knight A. R. and Woodruff G. N. (1988) [^{3}H]MK-801 labels a site on the *N*-methyl-D-aspartate receptor channel complex in rat brain membranes. *J. Neurochem.* **50,** 274.

Xu X. and Domino E. F. (1994) Phencyclidine-induced behavioral sensitization. *Pharmac. Biochem. Behav.* **47,** 603–608.